



Instructions for use

Imegen[®] MPL

Ref. IMG-236

CE IVD

Manufactured by:

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Health in Code, S.L. guarantees that all its products are free of defects in both materials and workmanship. This guarantee extends through to the expiry date, so long as the storage conditions described in this manual are observed.

Our products are intended for *in vitro* diagnostic use. Health in Code, S.L. provides no other guarantee, whether explicit or implicit, that extends beyond the proper functioning of the components of this kit. Health in Code, S.L.'s sole obligation, in relation to the aforementioned guarantees, shall be to either replace the product or reimburse the price thereof, at the client's choice, provided that however, materials or workmanship prove to be defective. Health in Code S.L. shall not be liable for any loss or damage, whether direct or indirect, resulting in economic loss or harm incurred as a result of use of the product by the buyer or user.

All the products marketed by Health in Code, S.L. undergo strict quality control. The **Imegen[®] MPL** kit has passed all internal validation tests, thus guaranteeing the reliability and reproducibility of each manufactured batch.

If you have any questions about the use of this product or its protocols, please contact our Technical Department:

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Imegen[®] is a trademark registered to Health in Code, S.L. in Spain.

Instructions for Use (IFU) modifications		
Version 11	DEC 2023	Review and update of section "3. Technical characteristics".
Version 10	DEC 2022	Modification of the storage and shipping temperature of the GENERAL MASTER MIX reagent (Section 4).
Version 09	NOV 2022	Change in manufacturer's address: Health in Code S.L., Calle de la Travesía s/n, 15E Base 5, Valencia 46024, Spain.
Version 08	SEP 2022	Change of the manufacturer's identification: from Imegen to Health in Code S.L.
Version 07	MAY 2021	Positive controls for targets W515L, W515K MPL and β -globin.
Version 06	AUG 2019	Enhanced analytical sensitivity (LOD 0.1%).
Version 05	APR 2019	Input DNA adjustment.

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01 General information

Myeloproliferative neoplasms (MPNs) are a group of chronic myeloid cell line cancer characterized by overproduction of precursors to mature blood cells.

The thrombopoietin receptor gene (*MPL* or *CD110*, located on 1p34) is mutated in 8–9% of essential thrombocythemia and primary myelofibrosis without mutations in *JAK2*, which is the main oncogenic event of this type of cancer. The thrombopoietin receptor is a 635-amino-acid transmembrane protein with two extracellular cytokine receptor domains. Upon thrombopoietin binding, *MPL* forms homodimers, activating the JAK–STAT pathway. The correct functioning of this pathway is crucial for the formation of megakaryocytes and platelets.

MPL mutations in MPNs specifically affect exon 10 codon 515, with W515K and W515L being the most frequent mutated forms.

References

- > Beer, P. A. et al. *MPL mutations in myeloproliferative disorders: Analysis of the PT-1 cohort. Blood* 112, 141–149 (2008).
- > Pikman, Y. et al. *MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med.* 3, 1140–1151 (2006).

02 Intended use

The Imegen® MPL kit tests for the W515L (NM_005373.2:c.1544G>T) and W515K (NM_005373.2:c.1543_1544delTGinsAA) mutations in the *MPL* gene in a qualitative real-time PCR assay.

This assay employs a combination of oligonucleotides and fluorescent hydrolysis probes in a validated assay to detect the presence of mutations in the *MPL* gene together with amplification of the endogenous gene, *β-globin*, in a genomic DNA sample. Detection of the *β-globin* gene acts as an internal positive control for the PCR reaction.

The results obtained by this assay can be used to confirm the diagnosis of the patient. This assay is not optimal for the study of minimal residual disease (MRD) in AML patients.

The Imegen® MPL kit is intended for *in vitro* diagnostic use only and is aimed at professionals in the field of molecular biology.

03 Technical characteristics

The **Imegen® MPL** kit has been validated using reference material obtained from the INCLIVA biobank, together with samples from previously diagnosed patients provided by the *Carlos Haya Regional University Hospital* (Málaga, Spain) and internal material of the Health in Code, S.L. Medical Genetics Unit, as well as certified synthetic vectors (GenScript) containing the sequences of interest. This vector works as a positive control, to verify the correct functioning of the PCR system. This validation represents a robust and specific diagnostic method.

The sensitivity and specificity of the *MPL* detection system has been confirmed by *in silico* and empirical studies including wild-type samples and mutant samples previously genotyped using other techniques. The limit of detection (LOD) of the systems for the detection of the W515L and W515K mutations has been established at 1%.

The type of material needed for this assay is genomic DNA extracted from peripheral blood. The total amount required per assay is 100 ng.

04 Safety warnings and precautions

- ◇ Strictly follow the instructions of this manual, especially regarding the handling and storage conditions of the reagents.
- ◇ Do not mouth-pipette.
- ◇ Do not smoke, eat, drink, or apply cosmetics in areas where kits and samples are handled.
- ◇ Any skin conditions, cuts, abrasions, and other skin injuries must be properly protected.
- ◇ Do not pour the remains of reagents down the drain. It is recommended to use waste containers established by the legal norm and manage their treatment through an authorized waste management facility.
- ◇ In the event of an accidental spill of any of the reagents, avoid contact with the skin, eyes, and mucous membranes and rinse with abundant water.
- ◇ Safety data-sheets (MSDS) of all dangerous substances contained in this kit are available on request.
- ◇ This product requires the handling of samples and materials of human origin. You should consider all materials of human origin as potentially infectious and handle them according to level 2 of the OSHA norm on biosafety and bloodborne pathogens or other practices related to biosafety of materials that contain or are suspected to contain infectious agents.
- ◇ The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic, corrosive, or environmental biological pollutants.
- ◇ This kit has been validated with specific equipment and under specific conditions that may vary widely among laboratories. Therefore, it is recommended that each laboratory conduct an internal validation when the kit is to be used for the first time.
- ◇ The manufacturer assumes no responsibility for the malfunction of the assay when the reagents included in the kit are replaced with other reagents not provided by Health in Code, S.L.
- ◇ The manufacturer does not guarantee the assay's reproducibility when the user uses reagents that have not been validated by Health in Code, S.L but are considered by the user equivalent to those provided in the kit.

05

Content and storage conditions of the kit

The kit contains the following reagents required for 24 PCR reactions for each Master Mix:

- **Master Mix MPL (W515K/W515L):** specific Master Mix for each mutation. Each Master Mix contains the oligonucleotides and probes necessary for the amplification. Detection of the specific *MPL* mutation is done by a probe labeled with the FAM™ fluorochrome. In addition, both systems include a VIC™ dye-labeled probe for the specific detection of the endogenous gene, *β-globin*.
- **General Master Mix:** PCR Master Mix with the nucleotides, MgCl₂, enzyme, and buffer necessary for the real-time PCR.
- **Positive Controls (MPL W515K Control and MPL W515L Control):** positive controls containing the sequence for the amplification of the tested mutations, W515K and W515L.

Reagents	Color indicator	Amount	Storage
MPL W515K Master Mix	Red disk	180 µl	-20°C
MPL W515L Master Mix	Yellow disk	180 µl	-20°C
General Master Mix	White disk	600 µl	-20°C*
MPL W515K Control	Red cap	25 µl	-20°C
MPL W515L Control	Yellow cap	25 µl	-20°C

Table 1. Components of the Imegen® MPL kit

(*) General Master Mix: it should be kept frozen until first use, protected from light, and stored between 2-8 °C after first use.

06

Equipment, reagents and materials not included in the kit

Equipment:

- Real-time PCR thermocycler (with FAM and VIC channels)
- Micropipettes (10 µL, 20 µL and 200 µL)
- Vortex mixer
- Centrifuge

Reagents:

- Nuclease-free water

Materials:

- Optical 96-well plates or 0.2-mL tubes
- Optical consumables compatible with the real-time PCR thermal cycler
- Filter pipette tips (10 µL, 20 µL, and 200 µL)
- Sterile 1.5-mL tubes
- Dust-free gloves

Complementary kits

Health in Code, S.L. also offers the following real-time PCR assays for the diagnosis of hematopoietic neoplasms:

- Imegen® **NMP1** (ref.: IMG-235)
- Imegen® **BCR-ABL1 Screening** (ref.: IMG-108)
- Imegen® **Inv16** (ref.: IMG-109)
- Imegen® **M-BCR-ABL1** (ref.: IMG-121)
- Imegen® **m-BCR-ABL1** (ref.: IMG-122)
- Imegen® **PML-RARA** (ref.: IMG-111)
- Imegen® **PML-RARA Screening** (ref.: IMG-130)

Health in Code, S.L. also offers the following fragment analysis and capillary electrophoresis assays for the diagnosis of hematopoietic neoplasms:

- Imegen® **CALR** (ref.: IMG-237)
- Imegen® **FLT3** (ref.: IMG-238)

07 Assay protocol

07.1 | Preparation of the amplification reactions

The protocol for preparation of amplification reactions is showed below:

- 01 Thaw all reagents included in the kit and DNA from the samples.
- 02 Spin each reagent on a vortex mixer and keep cold.
- 03 In 1.5-mL tubes, prepare two PCR mixes as specified in the following table:

Reagent	Volume per reaction	
	MPL W515L Master Mix	MPL W515K Master Mix
MPL W515L Master Mix	7.5 µL	-
MPL W515K Master Mix	-	7.5 µL
General Master Mix	12.5 µL	12.5 µL

NOTE: to estimate the necessary amount of reagents, we recommend making the calculations by taking into account the number of samples and controls that are to be analyzed at the same time and adding 10% of each reagent for an extra reaction.

- 04 Vortex and spin the PCR master mixes and dispense 20 µL into each optical consumable well.
- 05 Once the PCR master mixes have been dispensed, add the following into the corresponding wells:
 - ◇ 5 µL of the gDNA sample (10 ng/µL)
 - ◇ 5 µL of each positive control
 - ◇ 5 µL of nuclease-free water (NTC)

NOTE: a negative PCR control should be used in each master mix to ensure the absence of contamination, as well as positive control in each master mix to ensure the correct functioning of the PCR reaction.

- 06 Place the PCR tubes or PCR plate on the real-time thermal cycler and set up the amplification programmed as detailed in the following section.

07.2 | Settings for the real-time PCR program

◇ Hydrolysis probe fluorophores:

Probe	Fluorophore	Genotyping	Quencher*
W515L-P	FAM™	W515L-P	MGB
W515K-P	FAM™	W515K-P	MGB
β-globin-P	VIC™	β-globin	MGB

Table 2. Hydrolysis probe information

(* In the StepOne PCR System (Thermo Fisher Scientific) this field should be filled as "None"

➤ 7500 Fast or StepOne Real-Time PCR system (Applied Biosystems)

- ◇ Experiment: Quantitation – Standard Curve
- ◇ Ramp rate: Standard
- ◇ Reaction volume: 25 µL
- ◇ ROX™ baseline reference: included
- ◇ Optimal program:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR	
No. of cycles	1 initial cycle	45 cycles	
		Denaturation	Annealing / Extension
Temperature	95°C	95°C	64°C
Time	10 minutes	15 seconds	1 minute*

Table 3. Optimal PCR program for 7500 FAST or StepOne Plus (Applied Biosystem)

(* Fluorescence detection)

➤ LightCycler 480 Real-time PCR System (Roche)

- ◇ Experiment: Dual Color Hydrolysis Probe / UPL Probe
- ◇ Reaction volume: 25 µL
- ◇ Analyses: Abs Quant / Fit Points
- ◇ Optimal PCR program:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR		
No. of cycles	1 initial cycle	45 cycles		
		Denaturation	Annealing	Extension
Temperature	95°C	95°C	64°C	72°C
Time	10 minutes	15 seconds	30 seconds*	30 seconds

Table 4. Optimal PCR program for the LightCycler 480 PCR System (Roche)

(* Fluorescence detection)

08 Analysis of results

The following recommendations should be followed to ensure an adequate analysis of results:

- ◇ Make sure that no amplification occurred in negative PCR controls, either in the FAM or in the VIC channels. If amplification is detected, it is recommended to repeat the assay to rule out accidental contamination.
- ◇ Make sure that the *MPL* mutations—W515K or W515L, depending on the employed Master Mix (FAM channel)— and the reference gene—*β-globin* (VIC channel)—amplify in the positive controls
- ◇ Make sure that amplification of the *β-globin* gene (VIC) occurred in all analyzed samples. No visible amplification of *β-globin* is an indicator of low-quality DNA from the sample, preventing any conclusions regarding the studied samples to be drawn.
- ◇ Proprietary software of the used real-time PCR system must be used to analyze the samples. It is recommended to set up the threshold at 0.1 on thermal cyclers by Applied Biosystems and fix the noise band manually above the residual fluorescent signal on thermal cyclers by Roche.

The possible results obtained using the Imegen® MPL kit are shown below:

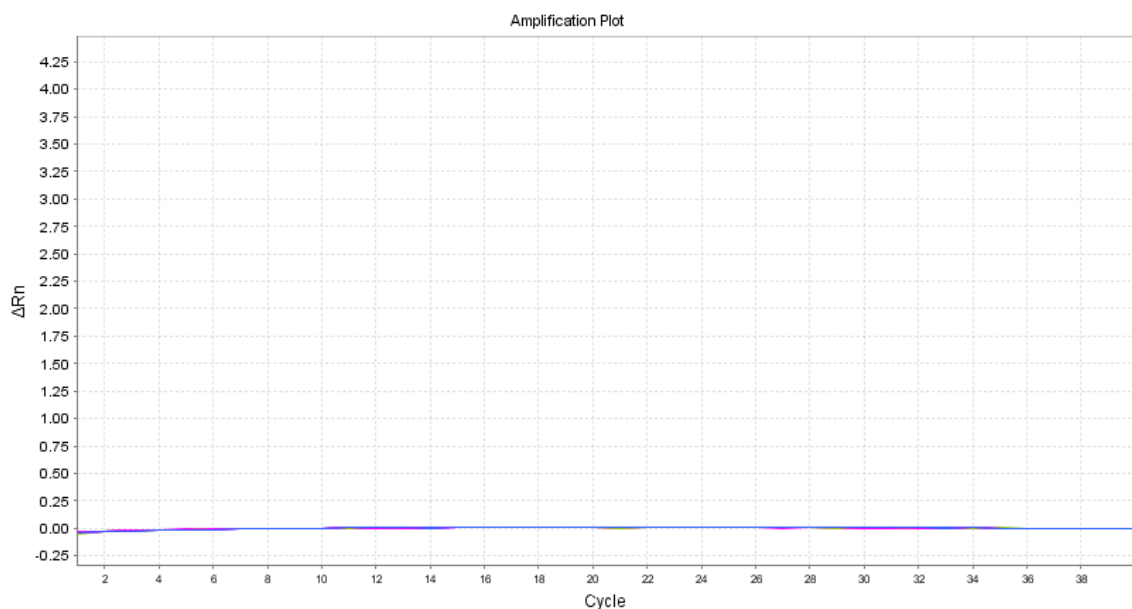


Figure 1. Expected result for PCR's negative control (NTC). No amplification is detected in the FAM or VIC channels.

W515K PCR System

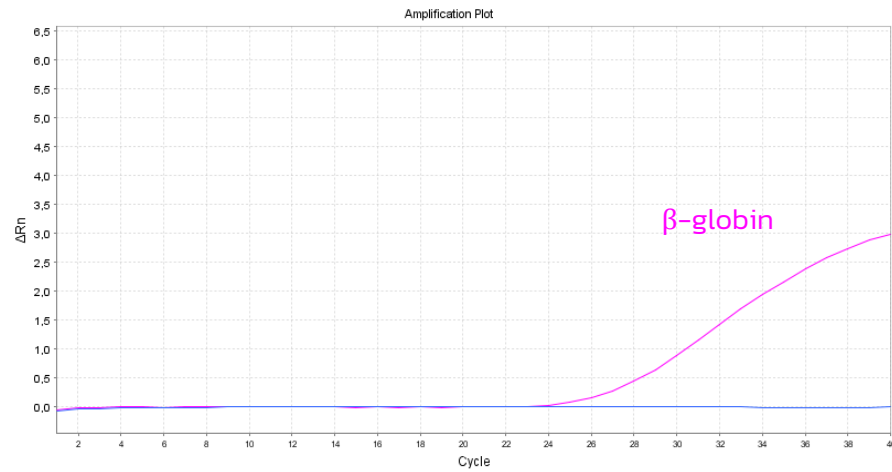


Figure 2. Result obtained from a DNA sample without any target mutation in the system. Amplification is detected only in the VIC channel (β -globin, shown in pink)

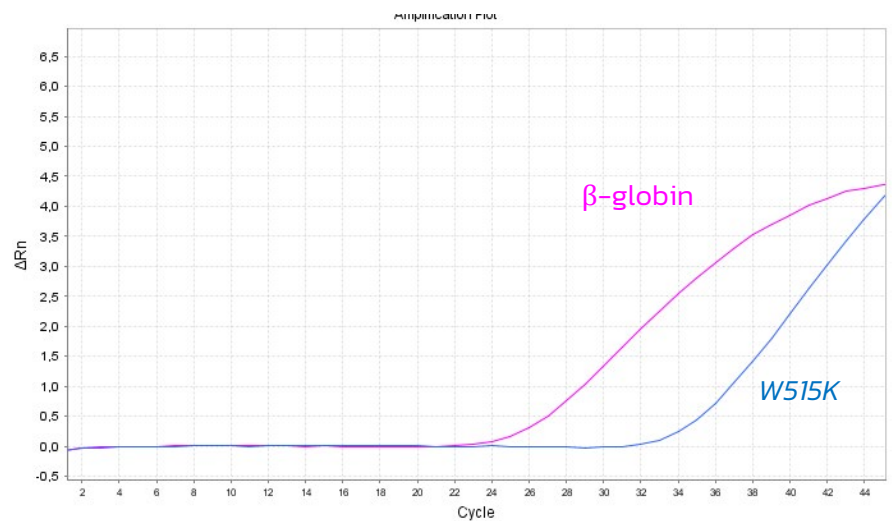


Figure 3. Results obtained from a sample with 1% of the MPL gene mutated (W515K). Amplification is detected in both fluorescent channels (in this figure, β -globin is shown in pink and MPL-W515K in blue)

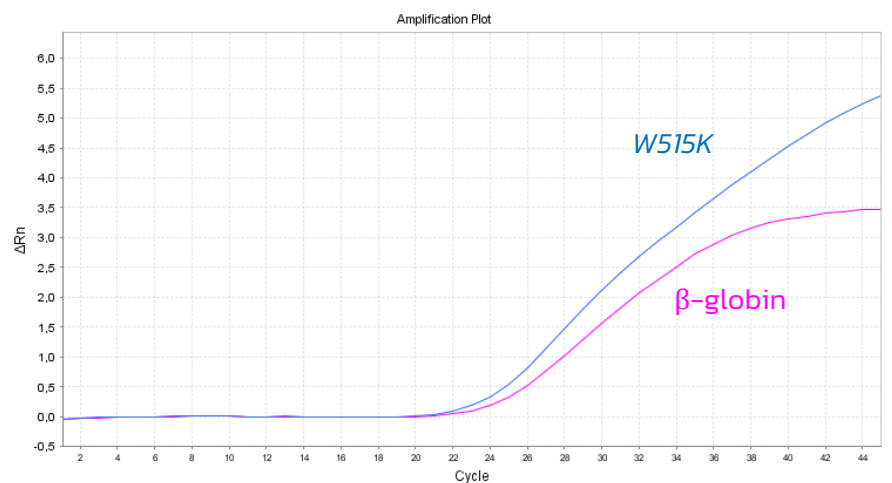


Figure 4. Expected results for the positive control, MPL-W515K amplification is shown in blue

W515L PCR System

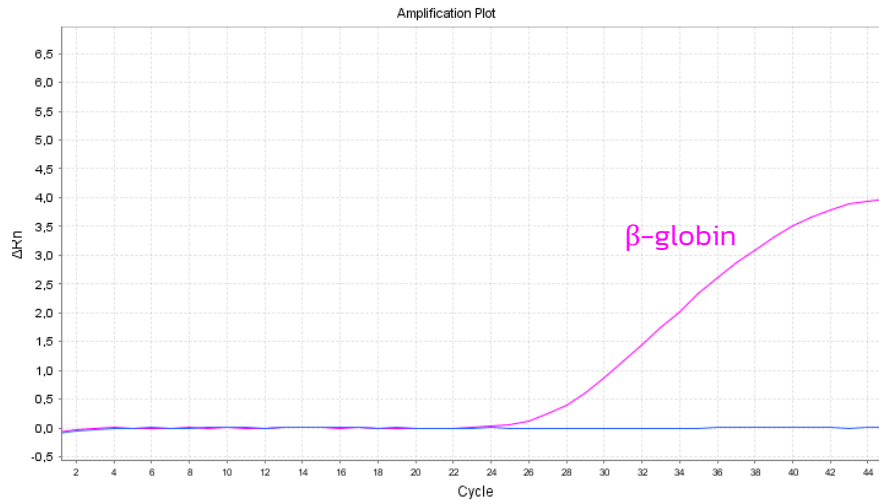


Figure 5. Results obtained from a DNA sample without any target mutation in the system Amplification is detected only in the VIC channel (β -globin, shown in pink)

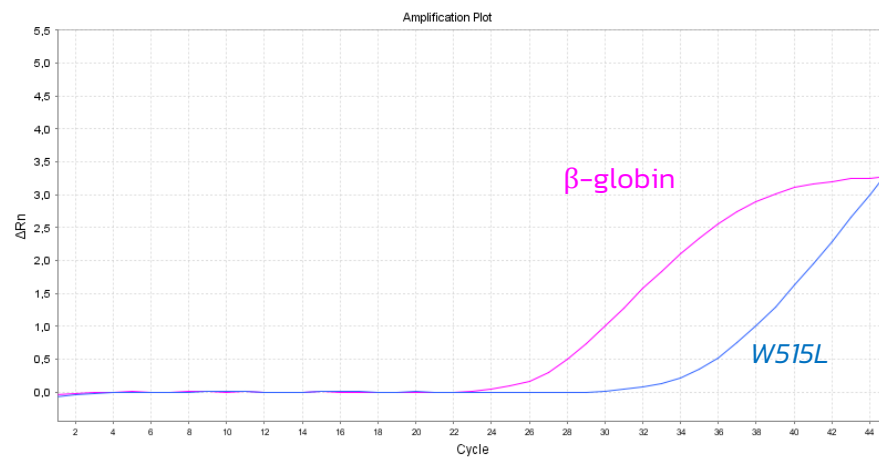


Figure 6. Results obtained from a sample with 1% of the MPL gene mutated (W515L). Amplification is detected in both fluorescent channels (in this figure, β -globin is shown in pink and MPL-W515K in blue)

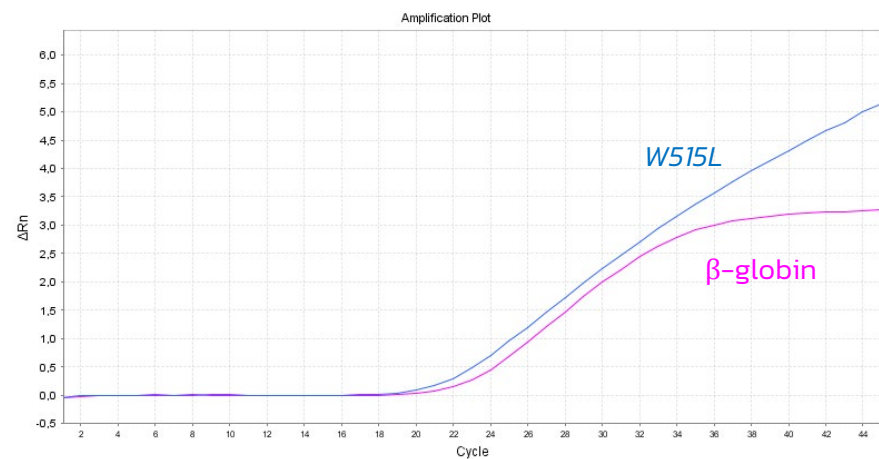


Figure 7. Expected results for the positive control, MPL-W515L amplification is shown in blue

09 Troubleshooting

The table below shows the results that could be obtained from the positive and negative controls and the DNA samples. If an unexpected result is obtained, the interpretation and the most likely reason for the result are given in the following table.

Control	MPL	β -Globin	Result / Interpretation
Positive controls	+	-	Expected result
	-	-	Failure in the PCR setup ¹
DNA sample	-	+	Expected result
	+	+	
	-	-	DNA sample failed to amplify ²
Negative control (NTC)	-	-	Expected result
	+	+	Contamination with human DNA or with the positive control ³

Table 4. Interpretation of the possible results obtained using Imegen[®] MPL

(1) **Failure in the PCR setup:** an error in the amplification may occur due to a technical issue during the PCR system setup. Check that the amplification program and the fluorescence detection setup are appropriate.

(2) **DNA sample failed to amplify:** an error to amplify the reference gene in the DNA sample may indicate that the quantity or the quality of the DNA sample is compromised. In this situation, a second analysis or DNA extraction from another sample would be recommended before repeating the assay and interpreting the results.

(3) **Contamination with human DNA or with the positive control:** PCR contamination may be caused by an inappropriate handling of the sample, the use of contaminated reagents, or be due to environmental contamination. To solve this issue, a thorough cleaning of the laboratory where the PCR reactions are prepared, including the equipment and material used, is recommended. If necessary, use fresh aliquots of the PCR reagents and prepare the PCR reactions containing the positive controls last in order to avoid any cross contamination.

10 Limitations

10.1 | Equipment

Imegen® MPL has been validated using the following PCR thermal cyclers:

- + *7500 FAST Real-Time PCR System* (Thermo Fisher Scientific)
- + *StepOne Real-Time PCR System* (Thermo Fisher Scientific)
- + *LightCycler 480 Instrument II* (Roche Life Science)

If a different brand or model of thermal cycler is used, the amplification program may need to be adjusted. Should you need further information or advice, please contact our technical support service.

10.2 | Reagents

Imegen® MPL has been validated using the reagents included in the kit and those recommended in section 6 of this document (Equipment, reagents and material not included in the kit).

10.3 | Product stability

The optimal performance of this product is achieved provided that the specified recommended storage conditions are applied, within the optimal product expiration date associated with each production batch.

Contact our Technical Department for any questions about the applications of this product or its protocols:

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Find out about all
our **diagnostic kits**

